Supplementary Materials for

Retraction of DNA-bound type IV competence pili initiates DNA uptake during natural transformation in *Vibrio cholerae*

Courtney K. Ellison[1], Triana N. Dalia[1], Alfredo Vidal Ceballos[3], Joseph Che-Yen Wang[2], Nicolas Biais[3], Yves V. Brun[1], and Ankur B. Dalia[1*].

[1]Department of Biology, Indiana University, 1001 E. 3rd Street, Bloomington, IN 47405.

[2]Electron Microscopy Center, Indiana University, Bloomington, IN 47405.

[3]Biology Department, CUNY Brooklyn College, 2900 Bedford Avenue, Brooklyn, NY 11210 and Graduate Center of CUNY, 365 5th Avenue, NY 10016.

*Correspondence to: ankdalia@indiana.edu.

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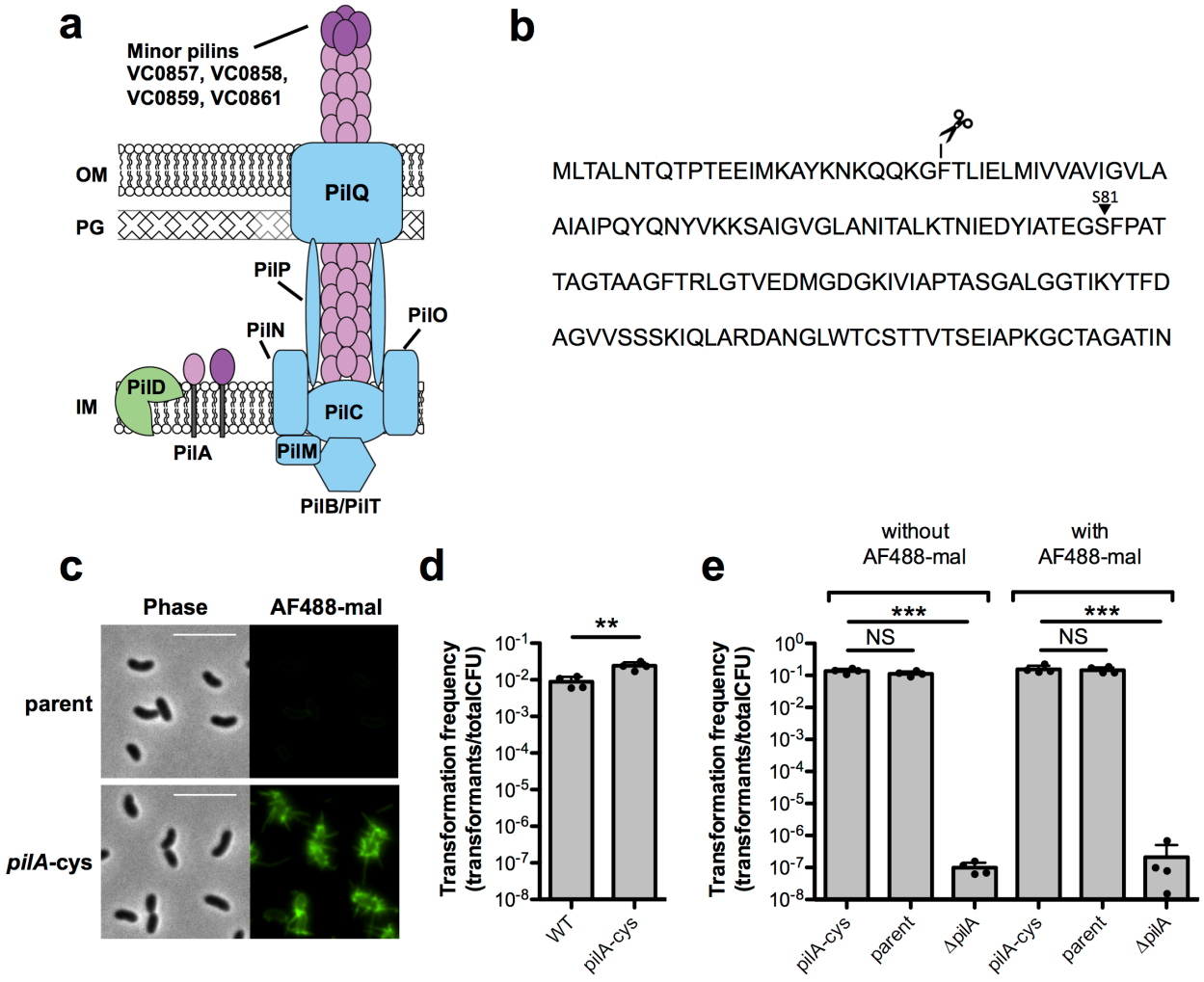
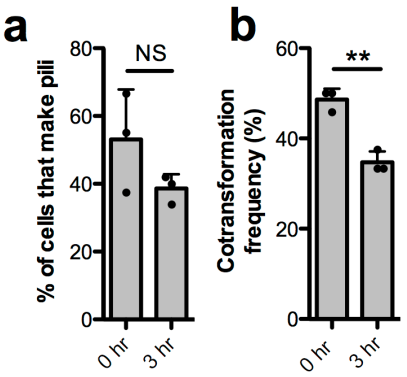
Supplementary Discussion

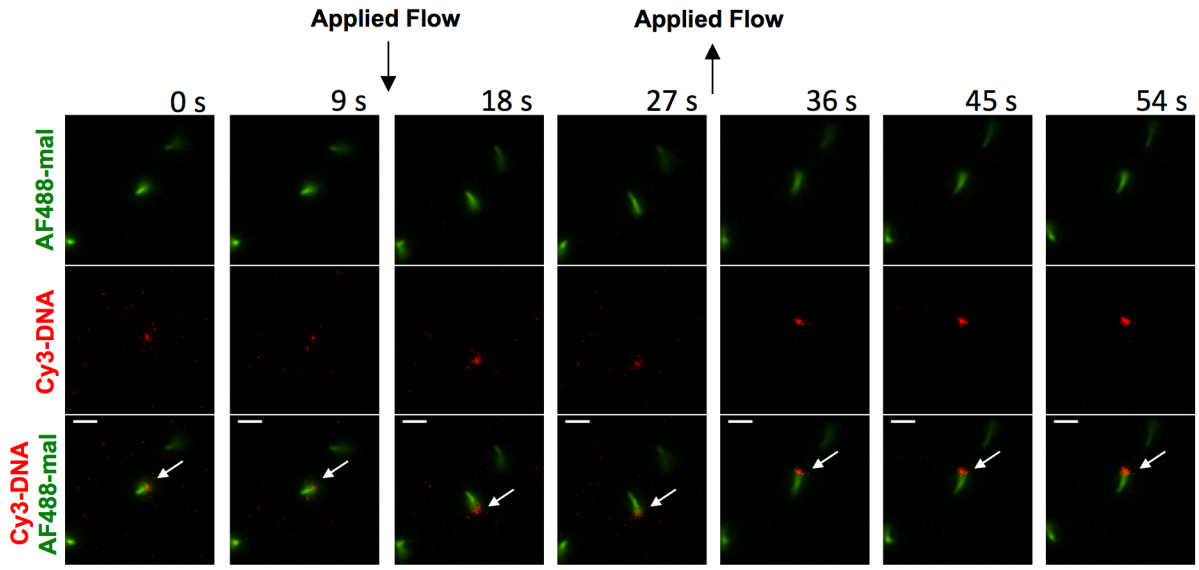
An interesting finding of this study is that *pilT* mutants are capable of retraction, and to some extent natural transformation. These results are at odds with earlier reports where *pilT* mutants exhibit transformation frequencies equal to a *pilA* mutant[7]. The discrepancy between the previously reported transformation frequencies and ours is likely due to differences in the strains and/or protocols used in each study. We constitutively induce competence in our study using a slightly different approach than that used by Seitz and Blokesch[7], which results in an ~2-3 log increase in the rates of natural transformation in our study. Competence is activated by both quorum sensing, which induces HapR expression, and chitin oligosaccharides, which induces expression of the competence regulator TfoX. In our study we constructed a constitutively competent strain by deleting *luxO* (to constitutively activate HapR expression) and by ectopically expressing the *tfoX* competence regulator (via an IPTG inducible P_{tac}-*tfoX* construct). Seitz and Blokesch[7] only overexpress *tfoX* to constitutively activate competence in their study (via an arabinose inducible *tfoX* construct). The ~2-3 log increase in transformation observed in our strain background increases the dynamic range of our transformation assays and likely allows us to observe transformants in mutant strains with very low transformation frequencies that are otherwise obscured as in the case of the *pilT* mutant. Another factor that may explain the differences observed is that each study uses different parental strain backgrounds of *V. cholerae*; while we use the El Tor strain E7946, Seitz and Blokesch[7] used the El Tor strain A1552. Thus, it is possible that strain to strain differences account for the difference in *pilT* phenotype observed between our studies.



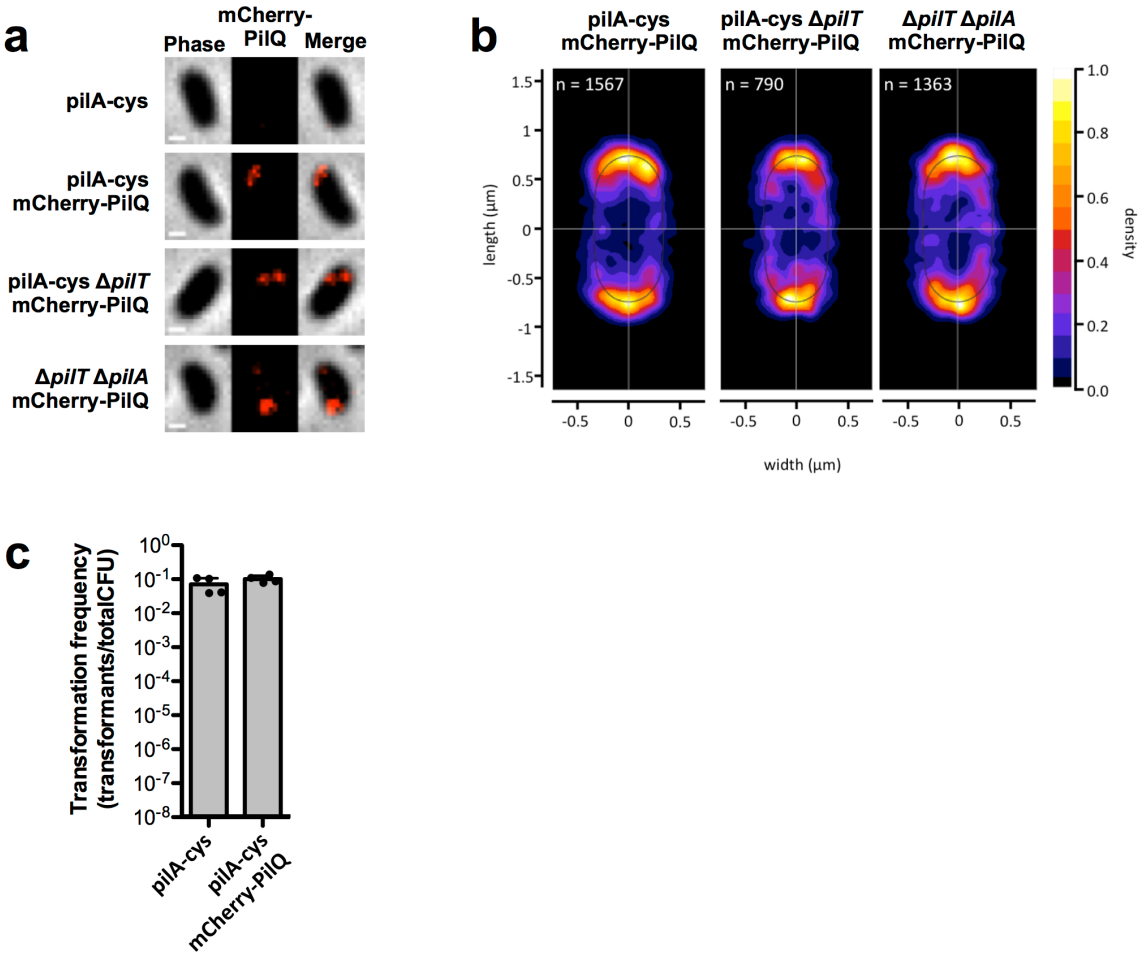
Supplementary Fig. 1. Introduction of a *pilA*-cys mutation into *pilA* allows for specific labeling of type IV competence pili without affecting their function. (**a**) Diagram of the competence pilus. Minor pilins were annotated based on the presence of the pilin motif GXXXXE. OM = outer membrane, PG = peptidoglycan, IM = inner membrane. (**b**) Primary sequence of PilA indicating the location of the *pilA*-cys mutation S81C. Scissors denote the prepilin cleavage site. (**c**) Static images of Δ*pilT* parent or *pilA*-cys cells labeled with AF488-mal dye. Scale bar, 5 µm. Images are representative of 3 independent experiments. (**d**) Chitin-dependent natural transformation assays of *pilA*-cys in a wildtype (WT) strain background (i.e. native *tfoX* and *luxO* intact). (**d**) Natural transformation assays of the indicated strains with and without AF488-mal in the transformation reaction. For **d** and **e**, cells were incubated with 500 ng of tDNA. Data from **d** and **e** are from four independent, biological replicates (n = 4 for all samples) and shown as the mean ± SD. Statistical comparisons were made by two-tailed Student's *t*-test. NS = not significant. ***P*<0.01, ****P* < 0.001.



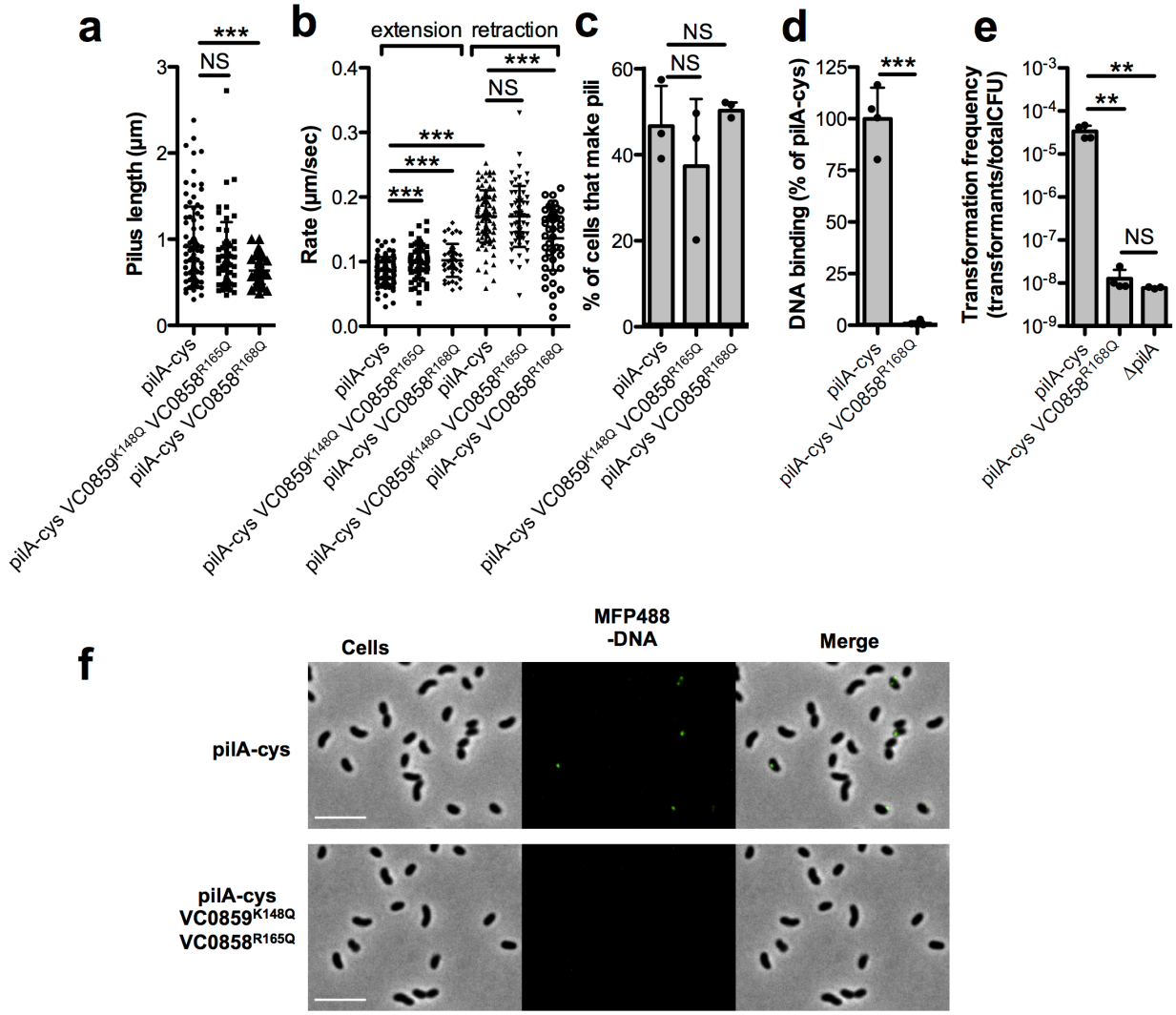
Supplementary Fig. 2. Cells remain competent for an extended period of time under the conditions tested. (**a**) *pilA*-cys cells were labeled with AF488-mal and analyzed by time-lapse microscopy to determine the number of pili made in each condition. Cells were either imaged immediately (0 hr) or after incubation under competence inducing conditions for 3 hr. Data are from three independent, biological replicates where a minimum of 50 cells were quantified. Percentage is the percent of cells within the population that make pili within a one-minute time-frame ± SD. (**b**) Natural transformation assays were performed where cells were incubated with two genetically unlinked markers (i.e. were cotransformed). One marker was added at time 0, and the second marker was added either immediately (0 hr) or 3 hours later (3 hr). Cells were then outgrown and plated to select for integration of the first marker and screened for integration of the second marker. The percent of cells containing both markers is reported as the cotransformation frequency. See Methods for details. All data are from three independent biological replicates and shown as the mean ± SD. Statistical comparisons were made by two-tailed Student's *t*-test. NS = not significant. $^{**}P < 0.01$.



Supplementary Fig. 3. Type IV competence pili bind DNA by their tip. Montage of time-lapse imaging of an additional sheared pilus from *pilA*-cys cells in a microfluidic channel after labeling with AF488-mal dye and mixing with Cy3-labeled DNA. The white arrows indicate DNA bound to a pilus over the course of the experiment. Black arrows above panels indicate the time and direction of flow that was applied through the microfluidic channel. Scale bar, 2 μm. Images are representative of three independent experiments.

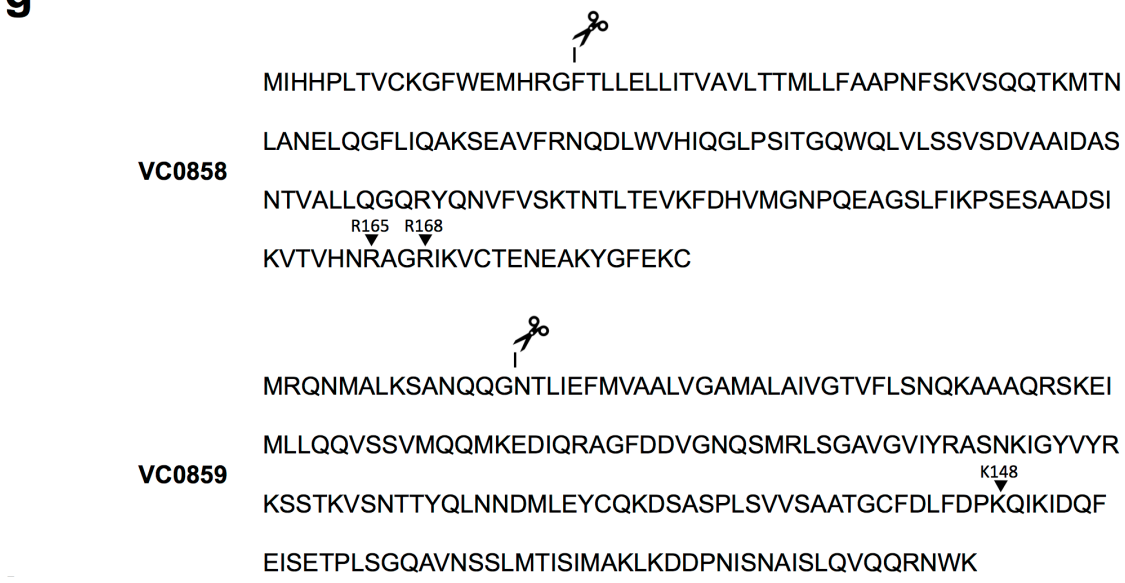


Supplementary Fig. 4. PilQ localizes normally in *pilA* and *pilT* mutants. (**a**) Static images of parent and mCherry-PilQ strains. Scale bar, 0.5 μm. (**b**) Heat maps of mCherry-PilQ localization in strains shown in **a**. Heat maps were generated by normalizing cell body lengths and plotting relative localization in three independent, biological replicates. (**c**) Natural transformation assays of the indicated strains where cells were incubated with 500 ng of tDNA, which demonstrates that the mCherry-PilQ construct is fully functional. Data are from four independent, biological replicates and shown as the mean ± SD.


a
b
c
d
e
Pilus length (μm)
Rate (μm/sec)
extension
retraction
% of cells that make pili
DNA binding (% of pilA-cys)
Transformation frequency (transformants/totalCFU)
NS
***
**
pilA-cys
pilA-cys VC0858R165Q
pilA-cys VC0858R168Q
pilA-cys VC0859K148Q VC0858R165Q
pilA-cys VC0859K148Q VC0858R168Q
ΔpilA

f
Cells
MFP488 -DNA
Merge
pilA-cys
pilA-cys VC0859K148Q VC0858R165Q

g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R165 R168
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MLLQQVSSVMQQMKEDIQRAGFDDVGNQSMRLSGAVGVIYRASNKIGYVYR
VC0859
K148
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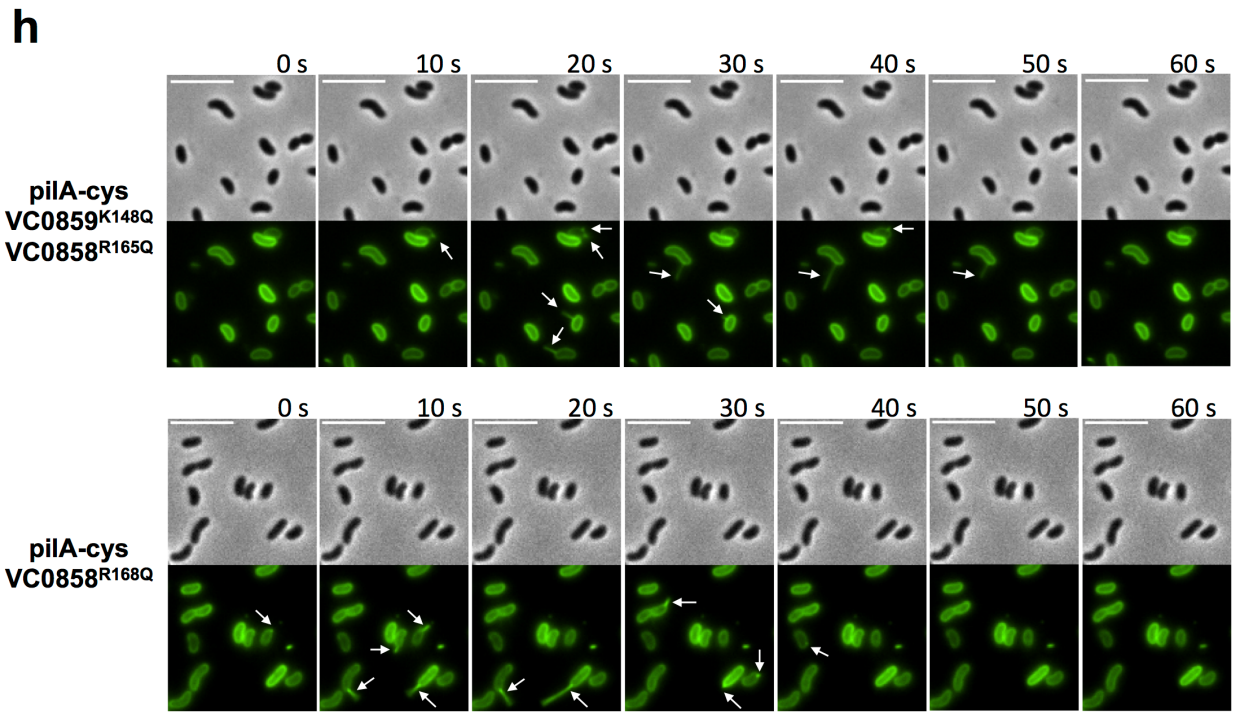
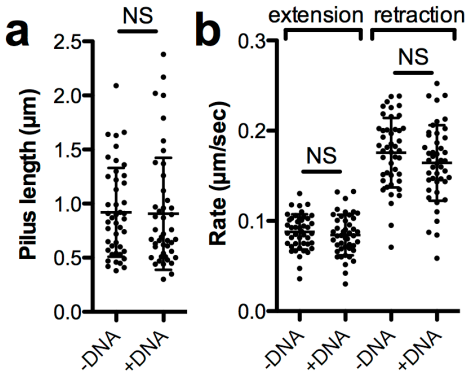
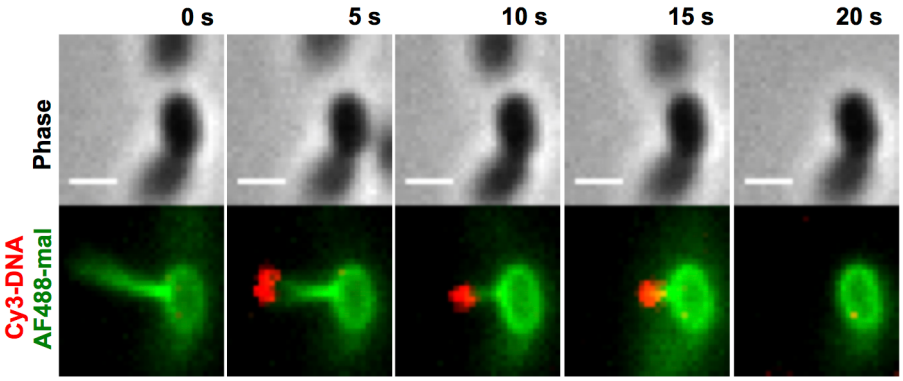


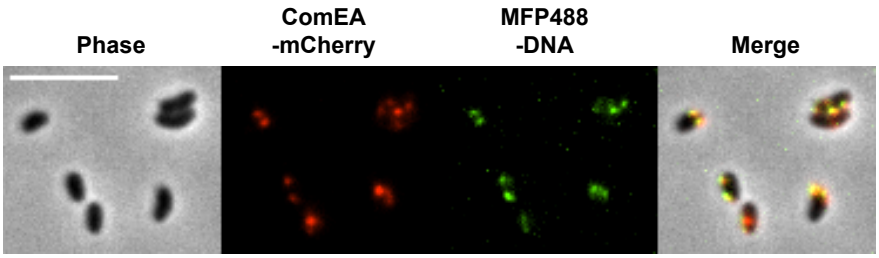
Supplementary Fig. 5. DNA-binding mutants display pilus dynamic activity similar to the *pilA*-cys parent. (**a**) Longest measured length of pili from cells that made a single pilus within a one-minute period for the indicated strains. Each data point represents the maximum length of an independent pilus. n = 45 for all strains. (**b**) Extension and retraction rates of pili in the indicated strains. Each data point represents the extension or retraction rate of an independent pilus. *pilA*-cys n = 90, piA-cys VC0859^{K148Q} VC0858^{R165Q} n = 60, *pilA*-cys VC1858^{R168Q} n = 45. (**c**) Percent of cells that make pili in populations of the indicated strains. Data are from three independent, biological replicates. (**d**) Relative DNA binding assay of the indicated strains in Δ*pilT* mutant backgrounds using a fluorescently labeled 6 kb PCR product. Data are from three independent biological replicates. (**e**) Natural transformation assays of the indicated strains. Cells were incubated with 5 ng of tDNA and DNase I was added to reactions after 10 mins to prevent additional DNA uptake. Data are from four, independent biological replicates. For **a**-**e**, all data are shown as the mean ± SD and statistical comparisons were made by two-tailed Student's *t*-test. NS = not significant. $^{**}P < 0.01$, $^{***}P < 0.001$. (**f**) Representative images of cells from the DNA internalization assay depicted in **Fig. 2f**. Scale bar, 5 µm. Images are representative of three independent experiments. (**g**) Primary sequence of the two minor pilins VC0858 and VC0859 indicating the location of the arginine and lysine mutations generated in this study. Scissors denote the prepilin cleavage site. (**h**) Montage of time-lapse imaging of *pilA*-cys VC0859^{K148Q} VC0858^{R165Q} or *pilA*-cys VC0858^{R168Q} cells after labeling with AF488-mal dye. The white arrows indicate pili. Scale bar, 5 µm. Images are representative of three independent experiments.



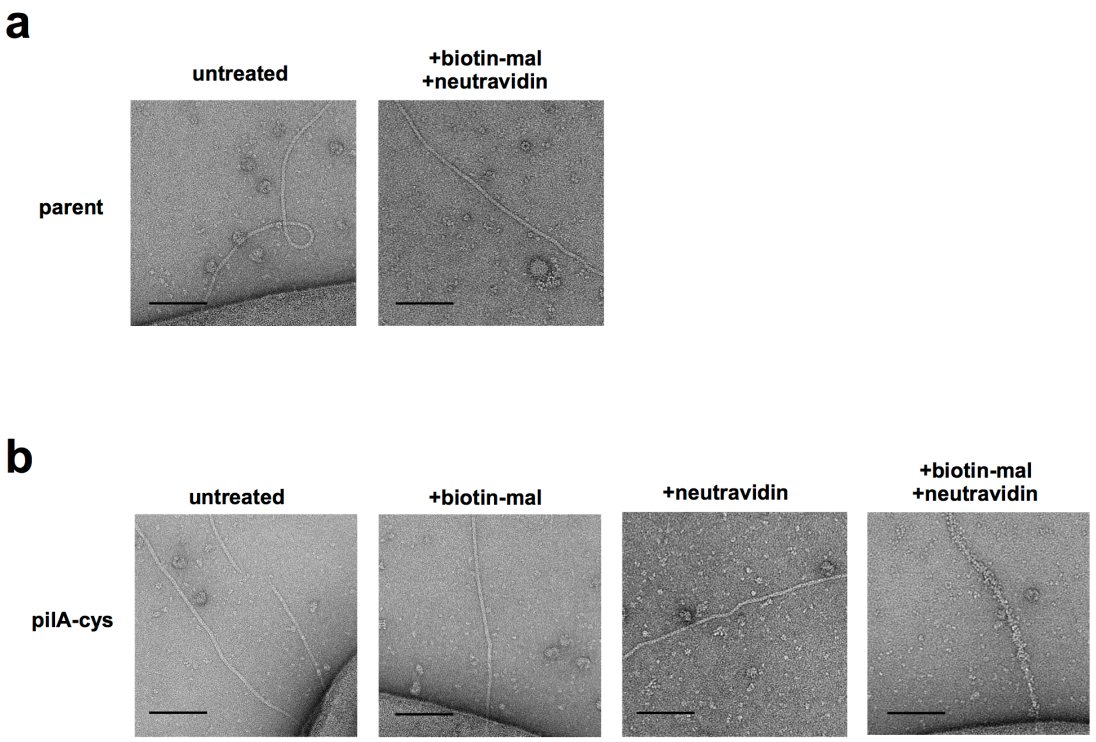
Supplementary Fig. 6. The presence of DNA does not alter pilus dynamics. *pilA*-cys cells were labeled with AF488-mal and subjected to time-lapse microscopy in the absence (-DNA) or presence (+DNA) of tDNA. Analysis revealed that the (**a**) pilus length and (**b**) extension and retraction rates were unaffected by the presence of DNA. Each data point represents an independent pilus. Data are shown as the mean ± SD and n = 45 for all samples. Statistical comparisons were made by two-tailed Student's *t*-test. NS = not significant.



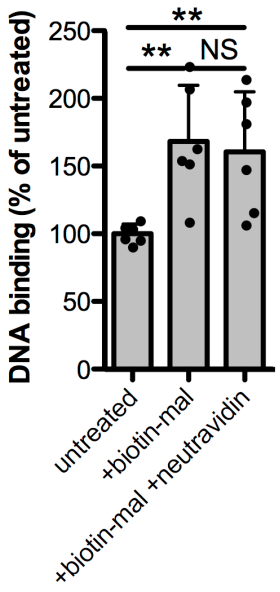
Supplementary Fig. 7. Type IV competence pili bring bound DNA to the cell surface. Montage of time-lapse imaging of *pilA*-cys strain after labeling with AF488-mal and incubation with Cy3-labeled DNA in a wet mount. Scale bar, 1 μm. Images are representative of three independent experiments.



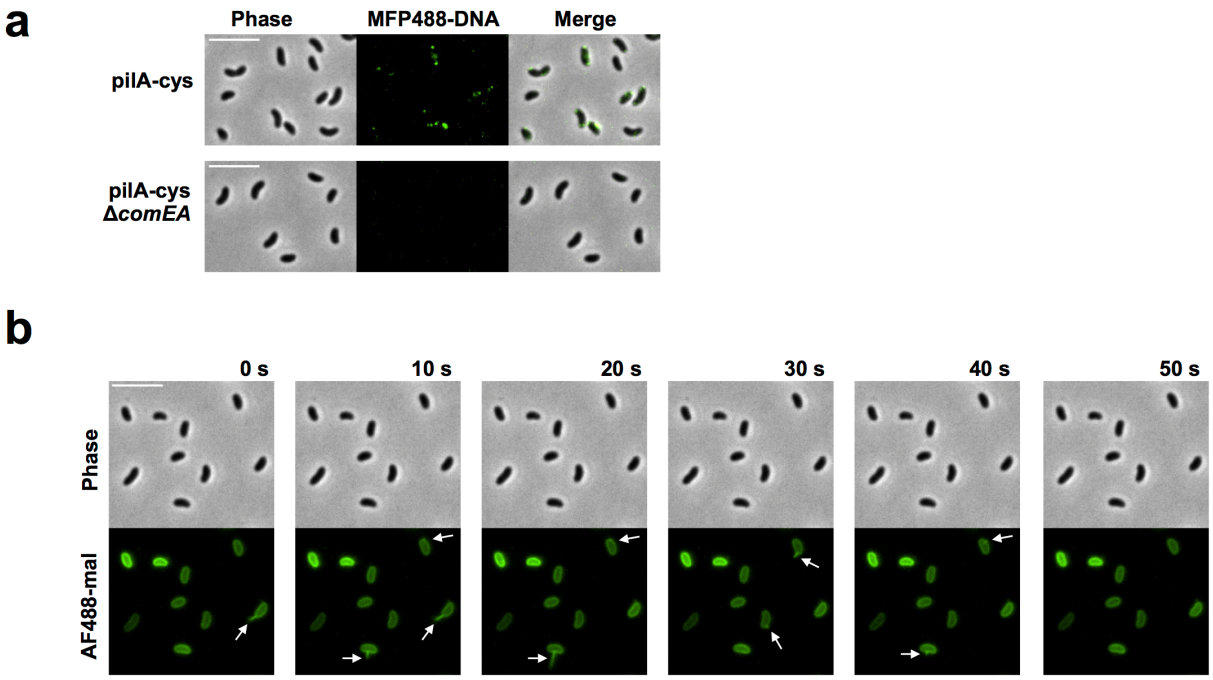
Supplementary Fig. 8. Fluorescently labeled DNA is taken up into the periplasm of cells and colocalizes with ComEA-mCherry foci. Static image of ComEA-mCherry strain after incubation with MFP488-labeled DNA. Scale bar, 5 μm. Images are representative of three independent experiments.



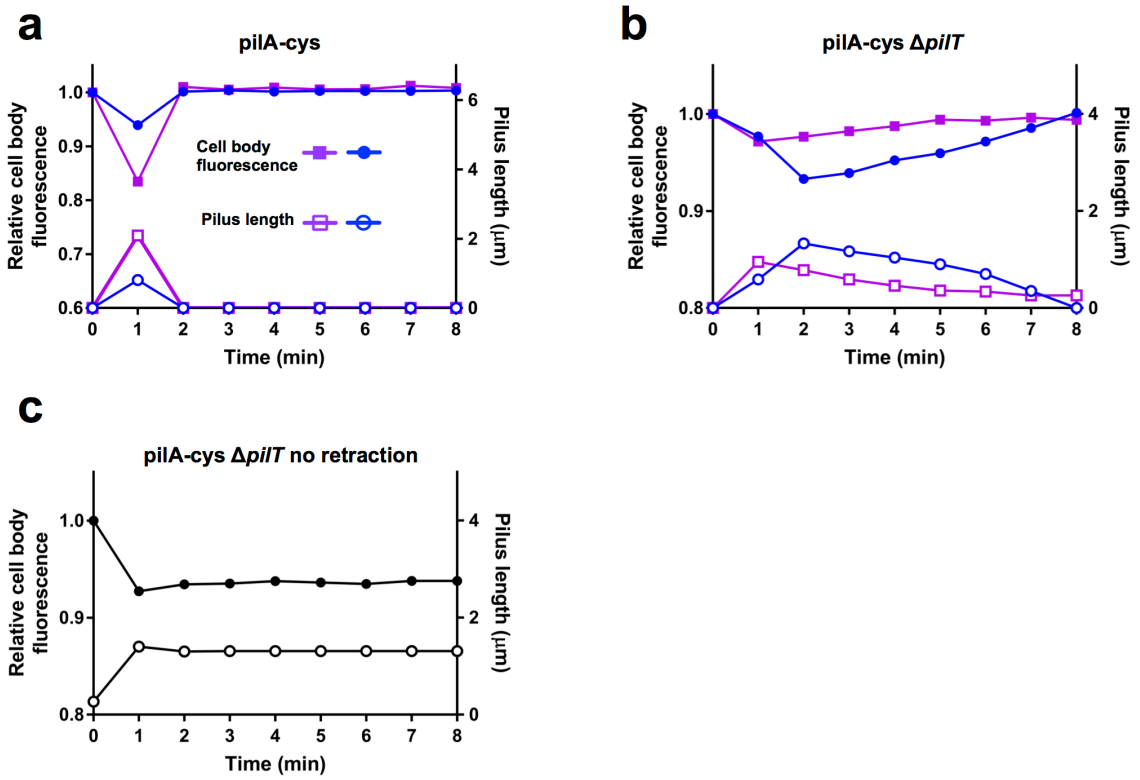
Supplementary Fig. 9. Steric obstruction of retraction via treatment with biotin-mal and neutravidin requires a *pilA*-cys mutation. Transmission electron micrographs of competence pili from (**a**) parent and (**b**) *pilA*-cys strains with indicated treatments. Strains for these experiments contained mutations to prevent production of other surface pili (ΔMSHA and ΔTCP), thus ensuring that the surface structures observed are competence pili. Scale bar, 100 nm. Images are representative of two independent experiments.



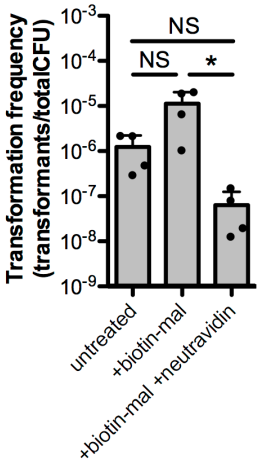
Supplementary Fig. 10. DNA binds normally to blocked pili. Relative binding of a Cy3-labeled 6 kb PCR product to *pilA*-cys Δ*pilT* cells following the indicated treatments. Data are from six independent, biological replicates and shown as the mean ± SD. Statistical comparisons were made by two-tailed Student's *t*-test. NS = not significant. $^{**}P < 0.01$.



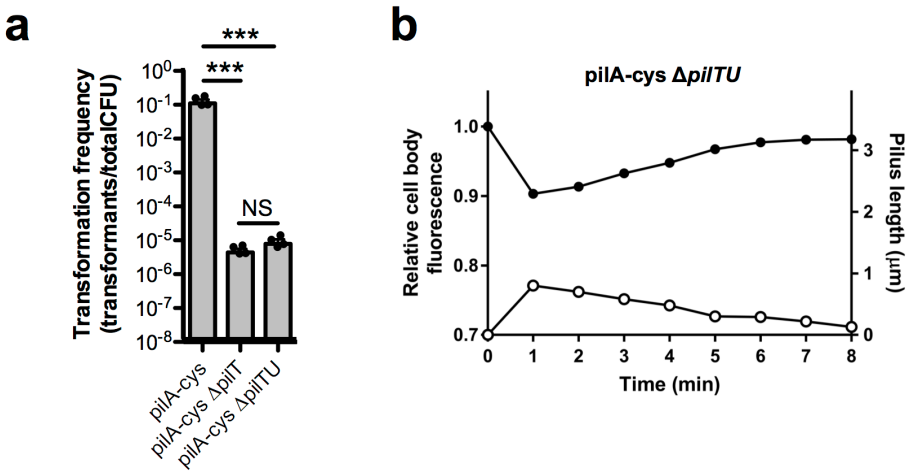
Supplementary Fig. 11. Uptake of DNA requires ComEA despite normal pilus dynamics in a *comEA* mutant. (**a**) Static image of the indicated strains after a one-hour incubation with 100 ng of MFP488-labeled 6 kb PCR product. Scale bar, 5 µm. (**b**) Montage of time-lapse imaging of *pilA*-cys Δ*comEA* cells after labeling with AF488-mal dye. The white arrows indicate pili. Scale bar, 5 µm. Images are representative of three independent experiments.



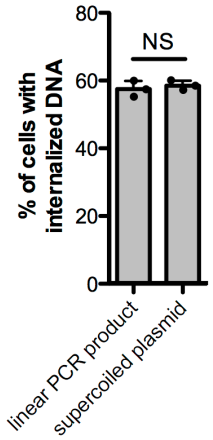
Supplementary Fig. 12. Measurement of retraction in *pilA*-cys and Δ*pilT* mutant cells. Plots showing relative cell body fluorescence (closed symbols) and correlated pilus length (open symbols) over time for additional (**a**) *pilA*-cys cells, (**b**) retracting *pilA*-cys Δ*pilT* cells, and (**c**) a *pilA*-cys Δ*pilT* cell that extended but never retracted a pilus, all labeled with AF488-mal. Colored lines (blue and purple) distinguish data from independent cells. Data are representative of three independent experiments.



Supplementary Fig. 13. Residual natural transformation in Δ*pilT* mutants requires retraction of surface exposed type IV competence pili. Natural transformation assays of *pilA*-cys Δ*pilT* cells after the indicated treatments. Cells were incubated with 500 ng of tDNA and DNase I was added after 1 hour to prevent additional DNA uptake. Data are from four independent, biological replicates and shown as the mean ± SD. Statistical comparisons were made by two-tailed Student's *t*-test. NS = not significant. **P* < 0.05.



Supplementary Fig. 14. The PilT homolog PilU does not promote PilT-independent retraction. (**a**) Natural transformation assays of the indicated strains. Data are from four independent, biological replicates and shown as the mean ± SD. Statistical comparisons were made by two-tailed Student's *t*-test. NS = not significant. ***$P < 0.001$. (**b**) AF488-mal labeled cells were subjected to time-lapse microscopy. Plots show relative cell body fluorescence (closed symbols) and correlated pilus length (open symbols) over time. Data are representative of two independent experiments.



Supplementary Fig. 15. Competent cells internalize both linear and circular DNA. DNA internalization assays were performed where *pilA*-cys cells were incubated with 10 ng of either an MFP488-labeled linear PCR product or circular supercoiled plasmid. Data are from three independent, biological replicates and shown as the mean ± SD. Statistical comparisons were made by two-tailed Student's *t*-test. NS = not significant**.**

Supplementary Table 1.
Strains used in this study.

Strain name in manuscript	Genotype and antibiotic resistances	Reference / (strain#)
parent	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*::$Spec^R$, *comEA*-*mCherry*, ΔVC1807::Cm^R	This study / TND0525 (SAD2154)
pilA-cys	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*::$Spec^R$, *comEA*-*mCherry*, ΔVC1807::Kan^R, *pilA* S81C	This study / TND0650 (SAD2155)
Δ*pilA*	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*::$Spec^R$, *comEA*-*mCherry*, ΔVC1807::Cm^R, Δ*pilA*	This study / TND0712 (SAD2156)
pilA-cys Δ*pilT*	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*::$Spec^R$, *comEA*-*mCherry*, ΔVC1807::Kan^R, *pilA* S81C, Δ*pilT*::Tm^R	This study / SAD2116
Δ*pilA* Δ*pilT*	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*::$Spec^R$, *comEA*-*mCherry*, ΔVC1807::Kan^R, Δ*pilA*, Δ*pilT*::Tm^R	This study / TND0854 (SAD2157)
WT	E7946 Sm^R	[31] / SAD030
pilA-cys (in WT background)	E7946 Sm^R, ΔVC1807::$Spec^R$, *pilA* S81C	This study / TND0852 (SAD2158)
parent Δ*pilT*	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*::$Spec^R$, ΔVC1807::Kan^R, Δ*pilT*::Tm^R	This study / TND0853 (SAD2159)
pilA-cys Δ*pilT*	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*::$Spec^R$, ΔVC1807::Cm^R, *pilA* S81C, Δ*pilT*::Tm^R	This study / TND0848 (SAD2160)
Δ*pilA* Δ*pilT*	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*::$Spec^R$, ΔVC1807::Cm^R, Δ*pilA*, Δ*pilT*::Tm^R	This study / TND0850 (SAD2161)
pilA-cys mCherry-PilQ	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*::$Spec^R$, ΔVC1807::Kan^R, *pilA* S81C, *mCherry-pilQ*	This study / TND0826 (SAD2162)
pilA-cys mCherry-PilQ Δ*pilT*	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*::$Spec^R$, ΔVC1807::Kan^R, *pilA* S81C, *mCherry-pilQ*, Δ*pilT*::Tm^R	This study / TND0857 (SAD2163)
mCherry-PilQ Δ*pilT* Δ*pilA*	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*::$Spec^R$, ΔVC1807::Kan^R, Δ*pilA*, *mCherry-pilQ*, Δ*pilT*::Tm^R	This study / TND0902 (SAD2164)
parent (for EM of pili)	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*, *comEA*-mCherry, ΔTCP::Zeo^R, ΔMSHA::$Carb^R$, ΔCTX::Kan^R, Δ*pilT*::Tm^R	This study / SAD2094
pilA-cys (for EM of pili)	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*, *comEA*-mCherry, *pilA* S81C, ΔTCP::Zeo^R, ΔMSHA::$Carb^R$, ΔCTX::Kan^R, Δ*pilT*::Tm^R	This study / SAD2093
pilA-cys Δ*comEA*	E7946 Sm^R, Δ*lacZ*::*lacIq*, P_{tac}-*tfoX*, Δ*luxO*::$Spec^R$, Δ*comEA*, ΔVC1807::Kan^R, *pilA* S81C	This study / SAD2138

pilA-cys VC0858^{R165Q}	E7946 Sm^{R}, Δ*lacZ*::*lacIq*, P$_{tac}$-*tfoX*, Δ*luxO*::Spec^{R}, *comEA-mCherry*, ΔVC1807::Cm^{R}, *pilA* S81C, VC0858 R165Q	This study / SAD2149
pilA-cys VC0859^{K148Q}	E7946 Sm^{R}, Δ*lacZ*::*lacIq*, P$_{tac}$-*tfoX*, Δ*luxO*::Spec^{R}, *comEA-mCherry*, ΔVC1807::Cm^{R}, *pilA* S81C, VC0859 K148Q	This study / TND0912 (SAD2166)
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pilA-cys VC0858^{R165Q} Δ*pilT*	E7946 Sm^{R}, Δ*lacZ*::*lacIq*, P$_{tac}$-*tfoX*, Δ*luxO*::Spec^{R}, *comEA-mCherry*, ΔVC1807::Cm^{R}, *pilA* S81C, VC0858 R165Q, Δ*pilT*::Tm^{R}	This study / SAD2151
pilA-cys VC0859^{K148Q} Δ*pilT*	E7946 Sm^{R}, Δ*lacZ*::*lacIq*, P$_{tac}$-*tfoX*, Δ*luxO*::Spec^{R}, *comEA-mCherry*, ΔVC1807::Cm^{R}, *pilA* S81C, VC0859 K148Q, Δ*pilT*::Tm^{R}	This study / TND0917 (SAD2167)
pilA-cys VC0858^{R165Q} VC0859^{K148Q} Δ*pilT*	E7946 Sm^{R}, Δ*lacZ*::*lacIq*, P$_{tac}$-*tfoX*, Δ*luxO*::Spec^{R}, *comEA-mCherry*, ΔVC1807::Cm^{R}, *pilA* S81C, VC0858 R165Q, VC0859 K148Q, Δ*pilT*::Tm^{R}	This study / SAD2152
pilA-cys VC0858^{R168Q}	E7946 Sm^{R}, Δ*lacZ*::*lacIq*, P$_{tac}$-*tfoX*, Δ*luxO*::Spec^{R}, *comEA-mCherry*, ΔVC1807::Cm^{R}, *pilA* S81C, VC0858 R168Q	This study / TND0971 (SAD2174)
pilA-cys VC0858^{R168Q} Δ*pilT*	E7946 Sm^{R}, Δ*lacZ*::*lacIq*, P$_{tac}$-*tfoX*, Δ*luxO*::Spec^{R}, *comEA-mCherry*, ΔVC1807::Cm^{R}, *pilA* S81C, VC0858 R168Q, Δ*pilT*::Tm^{R}	This study / TND0975 (SAD2175)
pilA-cys (micropillar assays)	E7946 Sm^{R}, Δ*lacZ*::*lacIq*, P$_{tac}$-*tfoX*, Δ*luxO*::Spec^{R}, ΔVC1807::Cm^{R}, *pilA* S81C, Δ*flaA*::Carb^{R}, Δ*mshA*, Δ*vps-rbmA*::Zeo^{R}, Δ*tcpA*::Kan^{R}	This study / TND0752 (SAD2171)
pilA-cys ΔpilT (micropillar assays)	E7946 Sm^{R}, Δ*lacZ*::*lacIq*, P$_{tac}$-*tfoX*, Δ*luxO*::Spec^{R}, ΔVC1807::Cm^{R}, *pilA* S81C, Δ*flaA*::Carb^{R}, Δ*mshA*, Δ*vps-rbmA*::Zeo^{R}, Δ*tcpA*::Kan^{R}, Δ*pilT*::Tm^{R}	This study / TND0767 (SAD2172)

Supplementary Table 2.

Primers used in this study.

Primer Name	**Primer Sequence (5'→3')**	**Description**
pilA S81C F	AAAACTAATATTGAAGATTATATTGCGACAGAAGGC TgTTTTCCTGCAACAACTGCAGG	*pilA* S81C F
pilA R2	CATTAATCGCGGTTTCAAAGTGCA	*pilA* R2
BBC374	TGGCAAAAAGCGAGAGAAGAAG	Δ*luxO* F1
BBC375	gtcgacggatccccggaatCATGAGGACATATTTTGTTTTCTGC	Δ*luxO* R1
BBC376	gaagcagctccagcctacaTAAGCGATGAGAGAATGGATCAAC	Δ*luxO* F2
BBC377	TCACACCCGAATTTCCATCATGC	Δ*luxO* R2
BBC1833	GATATCCATGTCCATGTCCCAG	*comEA*-mCherry or Δ*comEA* F1
BBC1834	cttgctcactccaccacttccacctgcTAACAAGATCCTTGCGGCATT G	*comEA*-mCherry R1
BBC1837	acgagctgtacaagtaaGCTGCAATCATGTGTCTCG	*comEA*-mCherry F2
BBC1838	GTCTTTCTATCAGTTCGGACTTAGTC	*comEA*-mCherry or Δ*comEA* R2
BBC1835	gcaggtggaagtggtggaGTGAGCAAGGGCGAGGAGG	mCherry (for comEA-mCherry) F
BBC1836	gattgcagcTTACTTGTACAGCTCGTCC	mCherry (for comEA-mCherry) R
BBC2249	gtcgacggatccccggaatTTGCATGATAGACCCTCATTTTGG	Δ*comEA* R1
BBC2250	gaagcagctccagcctacaTAAGCTGCAATCATGTGTCTCG	Δ*comEA* F2
ABD344	GATTAGCAACGATTCTAGCGCAGGAG	ΔVC1807 F1
ABD340	gtcgacggatccccggaatACGTTTCATTAGTCACCTCTATTGT TAACTTGTTC	ΔVC1807 R1
ABD341	gaagcagctccagcctacaTAGTCGAAAATAAAAAAAAGAGG CTCGCCTC	ΔVC1807 F2
ABD345	CTTGCTAACCGTTGGTGTTACCAGC	ΔVC1807 R2
BBC401	ACCAGCAAAGCTAATAAAATCGAG	Δ*pilA* F1
BBC402	gtcgacggatccccggaatGAGCATATGCCTTGCTACACAAG	Δ*pilA* R1
BBC403	gaagcagctccagcctacaACTGCAGGTGCAACAATTAACTAA	Δ*pilA* F2
BBC404	CGCCATACTAACCCAATACACTC	Δ*pilA* R2
DOG0400	ACTTCTGGCTGAAGGTCAATTTTC	Δ*pilT* F1
DOG0401	gtcgacggatccccggaatCATTTAAATTCCTTAATAAAGTCTG GC	Δ*pilT* R1
DOG0402	gaagcagctccagcctacaTAGGTAGGTAAAGACAGATGGAG	Δ*pilT* F2
DOG0403	TCACGTGTTCGGCCAAAATC	Δ*pilT* R2
BBC1904	TAGAGTGGCTACATCTGGCAG	mCherry-*pilQ* F1
BBC2145	cctcctcgcccttgctcacGGCGGTAGCGGATTCAGCAC	mCherry-*pilQ* R1
BBC2146	ggcaggtggagcaggtggaAACCAGTTGGAAAACATCGAC	mCherry-*pilQ* F2
BBC1907	TTCGTCCAATGCGTACAAGG	mCherry-*pilQ* R2
BBC2144	GTGAGCAAGGGCGAGGAGG	mCherry (for mCherry-pilQ) F
BBC1910	TCCACCTGCTCCACCTGCCTTGTACAGCTCGTCCATG C	mCherry (for mCherry-pilQ) R
BBC2123	TGATGGTAACTACTATAGGGTCG	ΔTCP locus F1

BBC2124	gtcgacggatccccggaatTAAATTAGGCTAGTGCCAGTCAG	ΔTCP locus R1
BBC2125	gaagcagctccagcctacaACAGGAGTTGCAGAAAAATAATGG	ΔTCP locus F2
BBC2126	ACTAAGATAATTGCTTCACGCATG	ΔTCP locus R2
DOG0436	AAGCGTTCTGGTTCAATCACC	ΔMSHA locus F1
DOG0434	gtcgacggatccccggaatCATTCTCTACCACTGCTATTTGGTTC	ΔMSHA locus R1
BBC2129	gaagcagctccagcctacaTAATGATTAAGCACTCAATGGATCCAG	ΔMSHA locus F2
BBC2130	TTTTGCATCAGCAAAATCACGC	ΔMSHA locus R2
BBC1443	GATTCAGACGGCAACTATCG	ΔCTX phage F1
BBC1444	gtcgacggatccccggaatGCGATTACACCATCAATCC	ΔCTX phage R1
BBC1445	gaagcagctccagcctacaGCACTAGGAACATTTTGTCTC	ΔCTX phage F2
BBC1447	CTGAAATGTGCTGTGTAAGC	ΔCTX phage R2
BBC2236	GGAATCTGCTGCAGATTCAATCAAAGTGACTGTGCACAATCaaGCTGGACGAATCAAAG	VC0858 R165Q F
BBC2240	TGTCTCTGCTGCGACAGGATGTTTTGATTTATTTGACCCGcAgCAAATCAAAATTGATC	VC0859 K148Q F
CE354	CTGCAGATTCAATCAAAGTGACTGTGCACAATCGGGCTGGACagATCAAAGTTTGCACC	VC0858 R168Q F
BBC1871	GTGAAGTAAAAGACGTTCTGC	VC0858 or VC0859 F1
BBC1875	AAAGCACAGCGTTCGTCC	VC0858 or VC0859 R2
BBC1450	CACTCAACGAGCTCAATACG	Δ*mshA* F1
ABD653	gtcgacggatccccggaatCATCTCTCTTTCATGTGAATACGCAGC	Δ*mshA* R1
ABD654	gaagcagctccagcctacaGCGCAATAATTTAAATATGGCTCGTGC	Δ*mshA* F2
BBC1453	ATAGCCTTGCTGTTCATTTTGG	Δ*mshA* R2
BBC2022	TGCCGATCTCGTTTATGGACG	Δ*vps-rbmA* locus F1
BBC2023	gtcgacggatccccggaatTGCCATTTTGATTGCCTCTGG	Δ*vps-rbmA* locus R1
BBC2024	gaagcagctccagcctacaCAAAGAGAGCCTTATTAGGCTCTC	Δ*vps-rbmA* locus F2
BBC2025	TGAAAGAGGTTGCTCTAGAACTCG	Δ*vps-rbmA* locus R2
BBC1752	CTGAATGATTTCCATGAGACG	Δ*flaA* F1
BBC1753	gtcgacggatccccggaatCATAGTTTGCTCTCCTATCGAG	Δ*flaA* R1
BBC1754	gaagcagctccagcctacaTTGCAGTAGTTCACGGTACCTTC	Δ*flaA* F2
BBC1755	TTATACGCTCTTTTGCGTGATGG	Δ*flaA* R2
BBC1457	TTTCAAGACTTTGGGCAATAG	Δ*tcpA* F1
BBC1968	gtcgacggatccccggaatCTGTTTTAATAATTGCATATTTATATAACTCCACC	Δ*tcpA* R1
BBC1969	gaagcagctccagcctacaTTTGGTAACAGTTAATCTACACCATTATCTTG	Δ*tcpA* F2
BBC1463	TTGAATTCGTCCATCATCTAAG	Δ*tcpA* R2

Supplementary Table 3

Complete statistical analysis.

Figure	Comparison	Statistical Test	P value	Significance
Fig. 1d	pilA-cys (n=7) vs parent (n=4)	Two-tailed Student's t-test	0.4572	NS
	pilA-cys (n=7) vs ΔpilA (n=7)	Two-tailed Student's t-test	<0.001	***
	Parent (n=4) vs ΔpilA (n=7)	Two-tailed Student's t-test	<0.001	***
Fig. 1e	none (n=7) vs +ssDNA (n=7)	Two-tailed Student's t-test	0.0106	*
	none (n=7) vs +dsDNA (n=7)	Two-tailed Student's t-test	<0.001	***
	none (n=7) vs +BSA (n=7)	Two-tailed Student's t-test	0.5980	NS
Fig. 2d	pilA-cys (n=4) vs pilA-cys VC0859^{K148Q} (n=4)	Two-tailed Student's t-test	<0.001	***
	pilA-cys (n=4) vs pilA-cys VC0858^{R165Q} (n=4)	Two-tailed Student's t-test	<0.001	***
	pilA-cys (n=4) vs pilA-cys VC0859^{K148Q} VC0858^{R165Q} (n=4)	Two-tailed Student's t-test	<0.001	***
	pilA-cys VC0859^{K148Q} (n=4) vs pilA-cys VC0859^{K148Q} VC0858^{R165Q} (n=4)	Two-tailed Student's t-test	<0.001	***
	pilA-cys VC0858^{R165Q} (n=4) vs pilA-cys VC0859^{K148Q} VC0858^{R165Q} (n=4)	Two-tailed Student's t-test	0.0044	**
Fig. 2e	pilA-cys (n=4) vs pilA-cys VC0859^{K148Q} (n=4)	Two-tailed Student's t-test	0.0062	**
	pilA-cys (n=4) vs pilA-cys VC0858^{R165Q} (n=4)	Two-tailed Student's t-test	0.0022	**
	pilA-cys (n=4) vs pilA-cys VC0859^{K148Q} VC0858^{R165Q} (n=4)	Two-tailed Student's t-test	0.0018	**
	pilA-cys VC0859^{K148Q} (n=4) vs pilA-cys VC0859^{K148Q} VC0858^{R165Q} (n=4)	Two-tailed Student's t-test	0.0013	**
	pilA-cys VC0858^{R165Q} (n=4) vs pilA-cys VC0859^{K148Q} VC0858^{R165Q} (n=4)	Two-tailed Student's t-test	<0.001	***
Fig. 2f	pilA-cys vs pilA-cys VC0859^{K148Q} VC0858^{R165Q} (n=3)	Two-tailed Student's t-test	<0.001	***
Fig. 3e	parent (n=3) vs parent +biotin (n=3)	Two-tailed Student's t-test	0.6890	NS
	parent (n=3) vs parent +biotin +neutravidin (n=3)	Two-tailed Student's t-test	0.2660	NS
	pilA-cys (n=3) vs pilA-cys +biotin (n=3)	Two-tailed Student's t-test	0.1153	NS
	pilA-cys (n=3) vs pilA-cys +biotin +neutravidin (n=3)	Two-tailed Student's t-test	<0.001	***
Fig. 4b	pilA-cys (n=4) vs pilA-cys ΔpilT (n=4)	Two-tailed Student's t-test	<0.001	***
	pilA-cys ΔpilT (n=4) vs ΔpilA (n=4)	Two-tailed Student's t-test	<0.001	***
	pilA-cys ΔpilT (n=4) vs ΔpilA	Two-tailed Student's t-test	<0.001	***

	ΔpilT (n=4)			
	ΔpilA (n=4) vs ΔpilA ΔpilT (n=4)	Two-tailed Student's t-test	0.1168	NS
Fig. 4c	pilA-cys (n = 79) vs pilA-cys ΔpilT (n = 339)	Two-tailed Student's t-test	<0.001	***
Fig. 4d	pilA-cys (n = 76) vs pilA-cys ΔpilT (n = 288)	Two-tailed Student's t-test	<0.001	***
Fig. S1d	WT (n = 4) vs pilA-cys (n = 4)	Two-tailed Student's t-test	0.0043	**
Fig. S1e	pilA-cys w/o AF488-mal (n = 4) vs parent w/o AF488-mal (n = 4)	Two-tailed Student's t-test	0.1753	NS
	pilA-cys w/ AF488-mal (n = 4) vs parent w/ AF488-mal (n = 4)	Two-tailed Student's t-test	0.7222	NS
	pilA-cys w/o AF488-mal (n = 4) vs ΔpilA w/o AF488-mal (n = 4)	Two-tailed Student's t-test	<0.001	***
	pilA-cys w/ AF488-mal (n = 4) vs ΔpilA w/ AF488-mal (n = 4)	Two-tailed Student's t-test	<0.001	***
Fig. S2a	0hr (n = 3) vs 3hr (n = 3)	Two-tailed Student's t-test	0.1765	NS
Fig. S2b	0hr (n = 3) vs 3hr (n = 3)	Two-tailed Student's t-test	0.0013	**
Fig. S5a	pilA-cys (n = 45) vs pilA-cys $VC0859^{K148Q}$ $VC0858^{R165Q}$ (n = 45)	Two-tailed Student's t-test	0.1206	NS
	pilA-cys (n = 45) vs pilA-cys $VC0858^{R168Q}$ (n = 45)	Two-tailed Student's t-test	<0.001	***
Fig. S5b	pilA-cys ext. (n = 90) vs pilA-cys $VC0859^{K148Q}$ $VC0858^{R165Q}$ ext. (n = 60)	Two-tailed Student's t-test	<0.001	***
	pilA-cys ext. (n = 90) vs pilA-cys $VC0858^{R168Q}$ ext. (n = 45)	Two-tailed Student's t-test	<0.001	***
	pilA-cys ext. (n = 90) vs pilA-cys ret. (n = 90)	Two-tailed Student's t-test	<0.001	***
	pilA-cys ret. (n = 90) vs pilA-cys $VC0859^{K148Q}$ $VC0858^{R165Q}$ ret. (n = 60)	Two-tailed Student's t-test	0.9956	NS
	pilA-cys ret. (n = 90) vs pilA-cys $VC0858^{R168Q}$ ret. (n = 45)	Two-tailed Student's t-test	<0.001	***
Fig. S5c	pilA-cys (n = 3) vs pilA-cys $VC0859^{K148Q}$ $VC0858^{R165Q}$ (n = 3)	Two-tailed Student's t-test	0.4268	NS
	pilA-cys (n = 3) vs pilA-cys $VC0858^{R168Q}$ ret. (n = 3)	Two-tailed Student's t-test	0.5487	NS
Fig. S5d	pilA-cys (n = 3) vs pilA-cys $VC0858^{R168Q}$ ret. (n = 3)	Two-tailed Student's t-test	<0.001	***
Fig. S5e	pilA-cys (n = 4) vs pilA-cys $VC0858^{R168Q}$ ret. (n = 4)	Two-tailed Student's t-test	0.0012	**
	pilA-cys (n = 4) vs ΔpilA (n = 4)	Two-tailed Student's t-test	0.0048	**
	pilA-cys $VC0858^{R168Q}$ ret. (n = 4) vs ΔpilA (n = 4)	Two-tailed Student's t-test	0.3202	NS
Fig. S6a	-DNA (n = 45) vs +DNA (n = 45)	Two-tailed Student's t-test	0.8890	NS
Fig. S6b	-DNA (n = 45) vs +DNA (n = 45) (extension)	Two-tailed Student's t-test	0.4411	NS
	-DNA (n = 45) vs +DNA (n = 45)	Two-tailed Student's t-test	0.1811	NS

	(retraction)			
Fig. S10	untreated (n = 6) vs +biotin-mal (n = 6)	Two-tailed Student's t-test	0.0027	**
	untreated (n = 6) vs +biotin-mal +neutravidin (n = 6)	Two-tailed Student's t-test	0.0079	**
	+biotin-mal (n = 6) vs +biotin-mal +neutravidin (n = 6)	Two-tailed Student's t-test	0.7661	NS
Fig. S13	untreated (n = 4) vs +biotin-mal (n = 4)	Two-tailed Student's t-test	0.0692	NS
	untreated (n = 4) vs +biotin-mal +neutravidin (n = 4)	Two-tailed Student's t-test	0.0573	NS
	+biotin-mal (n = 4) vs +biotin-mal +neutravidin (n = 4)	Two-tailed Student's t-test	0.0477	*
Fig. S14	pilA-cys (n = 4) vs pilA-cys ΔpilT (n = 4)	Two-tailed Student's t-test	<0.001	***
	pilA-cys (n = 4) vs pilA-cys ΔpilTU (n = 4)	Two-tailed Student's t-test	<0.001	***
	pilA-cys ΔpilT (n = 4) vs pilA-cys ΔpilTU (n = 4)	Two-tailed Student's t-test	0.0625	NS
Fig. S15	linear PCR product (n = 3) vs supercoiled plasmid (n = 3)	Two-tailed Student's t-test	0.5880	NS